Date April 25, 2000

MEMORANDUM

SUBJECT: ***Malathion*****:** Evaluation of the Cheminova Report Titled: A Pathology Working Group Review of Liver Slides from the 24-month Toxicity/Oncogenicity Study in the Rat

FROM: Marion Copley
RAB1, Health Effects Division (7509C)
and
Sanjivani Diwan
RRB4, Health Effects Division (7509C)

THROUGH: Alberto Protzel
TB1, Health Effects Division (7509C)

TO: Paula Deschamp
RRB2, Health Effects Division (7509C)
and
Patricia Moe
Reregistration Branch
Special Review and Reregistration Division (7508C)

ID: DP Bar code: D264515; Submission #: S529758, MRID 45069401 (PWG report)

This memorandum is an evaluation of the Pathology Working Group (PWG) report. The PWG report was provided to the Cancer Assessment Review Committee (CARC) as part of the package for presentation to the CARC on the April 12, 2000 meeting to reevaluate the carcinogenic potential of malathion. The CARC was to determine whether the liver tumor values from the Pathology Working Group (PWG) report should be used or those from the original study report. The CARC also considered the appropriateness of using the incidences of "hepatocellular alteration" as evidence of a possible pre-neoplastic response contiguous with adenoma responses. The CARC #2 final report will incorporate information from this memorandum, the PWG report as well as the recommendation from the HED consulting pathologist, and the CARC's determination.

The revised executive summary for "A 24-Month Oral Toxicity/Oncogenicity Study of Malathion (MRID 43942901)" is attached.

BACKGROUND

Cheminova A/S has submitted to the Agency (through Jellinek, Schwartz & Connolly, Inc.) three items:

1) A cover letter dated March 20, 2000, Malathion: "Pathology Working Group Review of Liver Slides from the 24-month Toxicity/Oncogenicity Study in the Rat (MRID 43942901);"

2) A "Summary and Significance of the Results from the Pathology Working Group Peer Review of Proliferative Lesions of the Liver in Female Rats in a 24-Month Oral Toxicity/Oncogenicity Study of Malathion (MRID 43942901)," dated march 20, 2000, no author is given;

3) A "Pathology Working Group (PWG) Peer Review of Proliferative Lesions of the Liver in Female Rats in A 24-Month Oral Toxicity/Oncogenicity Study of Malathion (MRID 43942901)," dated March 17, 2000.

While evaluating the PGW report several questions were raised by Brian Dementi and Marion Copley on April 7, 2000. These questions are listed in Attachment # 1 and were addressed by Dr. Jerry Hardisty, chairman, PWG, later during a meeting on April 10, 2000 (Attachment 2). Therefore, this evaluation of the PWG report also reflects the resolution of those issues. For further details refer to minutes of the meeting dated April 10, 2000.

GENERAL COMMENTS

1) The cover letter is a transmittal document stating that a PWG was conducted according to PR Notice 94-5 and why it was conducted. This was reaffirmed by the HED consulting pathologist, Dr. John Pletcher in a memorandum dated March 28, 2000 (Attachment 3) in response to questions raised by Brian Dementi (Attachment 4). The PWG reexamined the liver slides from all female rats with hepatocellular carcinoma, hepatocellular adenoma, hepatocellular hypertrophy/hyperplasia (regenerative), or moderate degrees of severity of hepatocellular alteration diagnosed either during the initial examination by the Study Pathologist or during the peer review by the Reviewing Pathologist.

   No evaluation of this is needed.

2) The Summary has two sections: Pathology Review and Reevaluation and Regulatory Implications of the PWG Report.

   This first part gives an **overview of the PWG and Peer Review report**. There are some oversimplifications and incorrect statements (i.e., "Furthermore, Cheminova agrees with CARC's conclusion that there was excessive toxicity at the 12,000 ppm dose level and that all tumors observed in this dose group should be disregarded for purposes of risk assessment."). Although CARC did say that there was excessive toxicity at the high dose, it **did not disregard this dose** for risk assessment purposes. It should be pointed out that denominators given in the tables for the controls and high doses include 15 animals from the 1 year sacrifice. The CARC does not include these animals in its calculations. Therefore, the CARC will base its

evaluation on the actual PWG and Peer Review report rather than the Summary. It is of interest that the Summary states in item 1, that Dr. Bolte was present at the pathology peer review (PPR) conducted by Dr. Busey while the PWG report itself does not mention his role in the PPR.

The CARC will independently determine the **regulatory implications** of the PWG report and not rely on the Summary for anything other than ideas for consideration.

3) The PWG and Peer Review (PWG) report will be used by the CARC to determine what tumor and non-neoplastic incidence values are most appropriate for liver tumors in the female rat liver. The PWG report presents information on the systemic toxicity present in the study and an interpretation of the significance of the tumors in light of this toxicity. However, like the information in the Summary, the CARC will not rely on this for anything other than ideas for consideration.

4) As stated by Dr. Hardisty, the signatures of the five PWG pathologists on page 5, indicate that they endorse the contents of the PWG report. The ancillary type of information, i.e. information beyond that of reading/diagnosing slides such as the historical control database and the other signs of toxicity in the study were included in the PWG report as referenced from the original study report. This information was discussed by the PWG and was included in the PWG report to provide support for the PWG's conclusions.

DISCUSSION OF THE PWG REPORT:

Both the Cheminova Summary and the PWG Report state that the review followed PR Notice 94-5 (which outlines the protocol for a PWG). This notice states that:

1) For any target tissue which is being re-evaluated, all slides containing this tissue in all groups must be re-read by a peer review pathologist.
2) The report from the study pathologist, peer review pathologist and original slides, are submitted to a PWG (similar to that described in the NTP Technical Reports).
3) The PWG is to review, at a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologists.
4) The PWG (according to the NTP Technical Reports) should consist of the peer review pathologist and other pathologists which includes the study pathologist (does not specify blind).
5) This group (according to the NTP Technical Reports) is to examine the chosen slides blind (no knowledge of dose groups or previously rendered diagnoses).
6) When the consensus differs (according to the NTP Technical Reports) from the study pathologist, the diagnosis is changed.
7) The PR notice goes on to say that the resulting report should include the PWG findings, the original diagnosis and the new diagnosis for each slide read. A comment column noting any discrepancies, missing slides, etc. should also included.

The PWG report states that:

a) The purpose of the peer review was to "validate the accuracy and consistency of the initial

histopathologic examination of tissues…." The reviewing pathologist read "all sections of liver from all female rats in all groups." It appears that the reviewing pathologist, Dr Busey, had an access to the doses and the diagnoses of the study pathologist. According to Dr. Hardisty, the peer review is not done in a coded manner. The primary purpose of the peer review conducted prior to a PWG review is to examine all slides of the organ being reviewed to identify any additional lesions which should be examined by the PWG and to confirm the findings reported by the study pathologist. The PWG report stated that the reviewing pathologist made an evaluation to validate the accuracy and consistency of the initial histopathologic examination of tissues and to employ current histopathologic criteria and nomenclature for proliferative lesions of the liver (Goodman et al., 1994) during the reexamination of the slides.

b) The results of the study pathologist (original diagnoses) and PPR were used by the PWG Chairperson in determining which slides were to be reviewed by the PWG.

c) The pathology peer review was conducted by. Dr. Busey on March 14, 2000. Following the peer review, the PWG Chairperson, Dr. Hardisty, examined the Individual Animal Gross Pathology findings from all female rats to identify female rats with a gross observation of liver nodule(s)/mass(es) noted at necropsy. The histopathology data were then reviewed from these animals to determine if a corresponding microscopic diagnosis was reported for these animals. Gross observations of nodule(s)/mass(es) were present for three Group I, four Group II, one Group IV and five Group V female rats. No corresponding lesions were present for nodule(s)/mass(es) noted grossly for one Group IV female, 4531, and one Group V female, 5512. The wet tissues were reexamined by the PWG Chairperson, Dr Hardisty and the study Pathologist, Dr. Bolte. The untrimmed lesions were sectioned by Dr. Bolte and the sections were processed. The slides were prepared and the material was organized including coding of slides for examination by the PWG the next day. Although the Summary of the PWG Report noted that Dr. Bolte was present at the peer review, the PWG Report did not clarify his role. However, according to Dr. Hardisty, Dr. Bolte was present but had no role in the Pathology Peer Review. As the study Pathologist, he made himself available to address any questions and retrieved any additional slides, reports, or tissues which were requested.

d) The PWG examined all sections with liver lesions including carcinoma, adenoma, hypertrophy/hyperplasia (regenerative), and moderate [or greater] degrees of severity of alteration diagnosed by either the study pathologist or the peer review pathologist. The PWG also examined sections where gross observations of nodule(s)/mass(es) were made in the liver. To this end, wet tissue from two animals (Group IV #4531 and Group V #5512) was resectioned because there were no correlating microscopic lesions to macroscopic observations. The report listed 3 group I, 4 group III, 1 group IV and 5 group V animals with nodule(s)/mass(es).

e) Members of the PWG included: Dr. Bolte (study pathologist), Dr. Busey (peer review pathologist), Dr. Hardisty (PWG Chairperson), and three other pathologists, Dr. Elwell, Dr. Hildebrandt and Dr. Garman. All of these are A.C.V.P. board certified and have many years experience evaluating rodent pathology.

f) The slides were coded prior to examination without knowledge of treatment group. Each pathologist noted his diagnoses and comments on worksheets. Each lesion was discussed and reexamined if needed.

g) The final opinions were recorded on the Chairperson's worksheets. A consensus diagnosis was considered when at least four of the five participants agreed. After the consensus diagnoses were recorded, the slides were decoded and the results tabulated by treatment group. "No changes were made to the consensus diagnoses after the slides were decoded by treatment group."

h) There was an Appendix (A) in the PWG Report comparing the diagnoses for individual animals from the study pathologist, peer review pathologist and the PWG. This Appendix included notes as to when there were no corresponding diagnoses. There was however, no comment column detailing what the differences in diagnoses were due to. There was a table comparing the summary tumor values from the study pathologist and the PWG (Table 2 in the PWG report). This also refers to Tables 1, 2 and 3 on pages 7 and 8 of this evaluation, respectively. Dr. Hardisty later on explained that a comment column was not included in the tabulation of the PWG findings in Appendix A of the PWG report. This was an oversight on his part. The comment column is intended to document discrepancies, missing slides, etc., rather than detail differences in opinion in diagnosis due to the application of diagnostic criteria. He further stated that no missing slides were noted during the review and the only discrepancies in the data noted during the review involved two untrimmed gross lesions discussed above, under item # c. Differences of opinion in diagnosis between the study pathologist's initial diagnoses tabulated in Appendix A resulted from the reexamination of the slides using the criteria included on pages 14 and 15 of the PWG report.

i) The pathology tables left certain things unexplained. For example, Drs. Bolte and Busey identified an adenoma for rat # 4531 in Group IV, while the PWG said "no corresponding diagnosis." It was unclear whether that meant they saw nothing, or "Hepatocellular alteration" encompassed the dual lesions identified by Drs. Bolte and Busey. Both Drs. Bolte and Busey identified an adenoma in Group V, rat # 5528, but the PWG disagreed with this diagnosis and reported "no corresponding diagnosis." Dr. Hardisty later explained that the PWG considered the lesion diagnosed initially as adenoma, to be a focus of cellular alteration (Attachment 2). Additionally, Drs. Bolte and Busey identified hepatocellular alteration in animal #5528. The PWG agreed with this diagnosis which was recorded across from the Study and Reviewing pathologists' findings.

j) Foci of cellular alteration are often considered pre-neoplastic lesions and part of the neoplastic continuum. The significance–relative to the carcinogenic potential–of the apparent increase in hepatocellular alteration over controls (1 in the controls as compared to 5-8 in all of the treatment groups) was unclear. The PWG did not specify as to the type of cellular alteration (basophilic or eosinophilic). In addition, the numbers are not representative of the total number of these foci since slides with slight or minimal alteration were not examined by the PWG unless selected due to another hepatic lesion. Dr Hardisty, during the meeting on April 10, 2000, explained that, in the opinion of the PWG, "hepatocellular alteration" should be regarded

as a nonneoplastic proliferative lesion of the liver. It should not be regarded as a preneoplastic lesion unless there is evidence of progression from hepatocellular alteration to neoplasia which was not evident in this study. He further stated that since the only purpose of the PWG was to confirm the incidence of hepatocellular neoplasm in female rats, no conclusions can be made concerning the incidence of hepatocellular alteration using only the data presented in the PWG Report.

For Discussion: There was good concordance between the diagnoses of Drs. Bolte and Busey. Of 13 tumor calls, they agreed on 11. In one case Dr. Busey identified adenoma in place of one carcinoma, and in the second case, down graded an adenoma to hepatocellular alteration. They both identified 12 tumors, and agreed on four of the five original carcinoma calls. Given the discrepancy between these initial two essentially concurring diagnoses and those of the final PWG, it would be informative to have more specific histopathologic information on what was seen microscopically that explains the revised diagnoses. This information should have been provided in a comments column. Also, the first two pathologists agreed on a tumor diagnosis, which was subsequently changed by the PWG, suggesting presence of a fine line of distinction.

It was noted in a memorandum by Dr. John Fletcher dated March 28, 2000, concerning the diagnosis of adenoma vs a focus of cellular alteration that both lesions can compress adjacent hepatic parenchyma, although adenoma typically does so much more than a focus, and can be composed of similar appearing cells forming a proliferative lesion of similar size. One person's adenoma may be another's focus of cell alteration. A consensus opinion of the PWG is considered more definitive.

CONCLUSIONS:

The PPR and PWG appeared to follow the procedure for a PWG as outlined in the PR notice for all steps as identified earlier. There were some questions raised in the above review which were clarified later by Dr. Hardisty. There was also an issue for discussion. Based on the Pathology Work Group (PWG) re-read, in female rats, there was a positive trend ($p=0.005$) for adenomas. The incidence of adenomas was significantly increased by pair-wise comparison at 12,000 ppm (5/38, 13%, $p=0.009$) when compared to controls (0/41). There were no carcinomas in any group. In the opinion of the PWG, "hepatocellular alteration" should not be regarded as a preneoplastic lesion unless there is evidence of progression from hepatocellular alteration to neoplasia which was not evident in this study. The CARC determined that the new tumors numbers should be used in the risk assessment for malathion. The CARC also determined that hepatocellular alteration did not appear to indicate progression to neoplasia. The CARC considered the new tumor numbers in conjunction with the remainder of the data base and determined that there was no indication of a tumorigenic response for liver tumors in the female Fischer 344 rat.

Table 1. Female Rat: Original Pathology Report, 1996 - Liver Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	0/40	1[a] /48	1/43	**3/39**	**3/29**
%	0	2	2	**8**	**10**
p=	**0.007****	0.240	0.168	**0.032***	**0.008****
Carcinomas	0/41	1/50	1/44	0/41	3[b] /38
%	0	2	2	0	8
p=	0.063	0.168	0.168	-	0.085
Combined	0/41	2/50	2/44	**3/41**	**6/38**
%	0	4	5	**7**	**16**
p=	**0.002****	0.134	0.085	**0.032***	**0.003****

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, July 16, 1997).

[a] First liver adenoma observed at week 103, dose 100/50 ppm.

[b] First liver carcinoma observed at week 101, dose 12,000 ppm

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then p <0.05; If **, then p <0.01

Table 2. Female Rats: PWG Re-read, 2000 - Liver Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas[a]	0/41	1/50	2/44	0/41	5/38[b]
%	0	2	5	0	13
p=	0.005**	0.168	0.085	–	0.009**

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Burnam, April 25, 2000).

[a] There were no carcinomas diagnosed at any dose.

[b] First liver adenoma observed at week 101, dose 12,000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then p <0.05; If **, then p <0.01

Table 3. <u>Female Rats:</u> Summary of the changes in tumor incidences between the original diagnosis, peer review pathologist and the PWG consensus

	Original Diagnosis			Peer Review Pathologist			PWG
Dose(ppm)	Adenoma	Carcinoma	Combined	Adenoma	Carcinoma	Combined	Adenoma
Control	0	0	0	0	0	0	0
100/50	1	1	2	1	1	2	1
500	1	1	2	1	1	2	2
6,000	3	0	3	2	0	2	0
12,000	3	3	6	4	2	6	5[a]

[a] Animal # 5512 had two adenomas

Statistics (Peto) for table 3 were made available at the CARC meeting.

ATTACHMENTS

1) List of questions regarding the PWG prepared on April 7, 2000 by Brian Dementi, TB1, Health Effects Division for a conference with Dr. Hardisty.

2) Minutes of the meeting with Dr. Jerry Hardisty on April 10, 2000.

3) Memorandum by John M. Pletcher, Consulting Pathologist, CARC Health Effects Division to Sanjivani Diwan, Executive Secretary, CARC, Health Effects Division, dated March 28, 2000.

4) Memorandum from Brian Dementi , TB1, Health Effects Division to Sanjivani Diwan, Executive Secretary, CARC, Health Effects Division, dated April 27, 2000.

5) Revised executive Summary of the DER for combined chronic/carcinogenicity study in rats (MRID 43942901).

Attachment 1

ATTENTION: Jerry Hardisty April 7, 2000

The following questions were the result of a discussion between Drs. Brian Dementi, Marion Copley and William Burnam (HED/OPP/EPA) regarding the Jellinek, Schwartz and Connolly, Inc. letter to Patricia Moe (March 20, 2000), their summary and the attached malathion PWG report for female rat livers.

We would like to have a conference call sometime early Tuesday morning (4/11/2000) if possible, between Drs. Brian Dementi, Marion Copley and William Burnam (HED/OPP/EPA), Judith Hauswirth (representing Jellinek) and Jerry Hardisty (EPL). Please call either Bill (703-305-7491) or Marion (703-305-7434) today (Friday, 4/7/2000) so we can set this up. The Cancer Assessment Review Committee meeting is on Wednesday (4/12/2000).

Questions regarding the PWG

1. **Differences** - The Jellinek, Schwartz and Connolly, Inc. letter to Patricia Moe (March 20, 2000) (includes Summary and Significance Results of .....) has several differences from their attached Pathology Working Group (report dated March 17, 2000).

a) The Summary states that "Dr. Henry Bolte, the original study pathologist, was present at the PPR," while the PWG report itself makes no mention of Bolte's presence at the PPR. Was Dr Bolte present and if Dr. Bolte was present on March 14, what was his role?

b) The Summary states in item 3. "The PWG was conducted in full compliance with the procedures described in PR Notice 94-5 (August 24, 1994). All slides containing sections previously diagnosed by the SP or the PPR as hepatocellular carcinoma or adenoma or as indicating **varying degrees of severity** (emphasis added) of non-neoplastic proliferative lesions (foci of cellular alteration and or hypertrophy/hyperplasia) were examined." (p. 1) By contrast, the PWG report says "hepatocellular alteration of **moderate degree of severity** (emphasis added) diagnosed by either the study pathologist or reviewing pathologist were submitted to the PWG." (p. 10) What was actually read by the PWG, all cellular alterations or only those diagnosed as of moderate severity or above by either the SP or PPR?

2. **Process questions**

a) The PWG abstract states, "The purpose of the PWG review was to determine the incidence of hepatocellular neoplasms in female rats following currently accepted nomenclature and diagnostic criteria." Do the pathologists' signatures on the PWG report endorse the contents of the entire report including the discussion of the historical control data base and the other signs of toxicity in the study in question OR do they just refer to the consensus report comparisons of the slides read by the PWG?

b) Did the chairperson complete the following after the peer review on 3/14/2000: 1) examined all slides that were positive, 2) have recuts made of two blocks, and 3) organized materials including coding slides for PWG on the 3/15/2000?

c) A peer review was performed on all slides. Is this review available to EPA upon request?

d) Can a reviewing pathologist reliably evaluate as many as an estimated 625 sections in one day?

e) What was the function of the chairman during the PWG reread?

f) What was the level of review (by the peer review pathologist) of all of the slides?

Was the evaluation made with the intent to just confirm the diagnoses of the study pathologist and identify any missed diagnoses; or did he independently determine his own diagnoses for all slides? Was his evaluation blind or did he have access to the existing diagnoses and the groups that the animals belonged to.

g) The PR notice states that "the Agency should be provided with a detailed pathology report, which presents the PWG findings and includes the original diagnosis and the new diagnosis of each slide read, and a comment column to note any discrepancies…" Is there a reason a comment column was not provided to explain where the differences came from?

3. **Technical questions**

a) Since hepatocellular alterations, hypertrophy/hyperplasia, adenoma and carcinoma constituted the focus of this PWG, and all of these end points play a role in characterizing the neoplastic response as discussed in Goodman, why was there no mention of the hepatocellular alterations in the discussion about carcinogenicity? The PWG addressed the interpretation of the adenoma and carcinomas in this study. Since hepatocellular alteration together with adenoma and carcinoma are said to constitute the natural history of neoplasia—although not required—it would have been informative to have the PWG's input into the interpretation based on incidences of all three of these end points.

b) In the case of a Group II rat #2554, a Group IV rat, #4531 and a Group V rat, #5528, both Drs. Bolte and Busey identified adenoma, while PWG reported "no corresponding diagnosis"; are we to assume they saw nothing, or perhaps a severe form of hepatocellular alteration?

c) If new criteria of classification are being applied in this study of malathion, what does the panel say regarding relevance of the historical control data base, from both NTP and the testing facility?

d) What was seen microscopically by the PWG that led to changes of diagnoses first rendered by Drs. Bolte and Busey? Also, to what extent do these revisions depend upon Goodman et al (1994)? There was no comment column detailing what the differences in diagnoses were due to.

e) Is one section of liver adequate to render a definitive diagnosis when there is a debate and change of diagnosis? In many cases Drs. Bolte and Busey were in agreement, but the diagnosis was changed by PWG, does this suggest that the lesions were borderline (e.g., between alteration-adenoma, adenoma-carcinoma)?

3. Cellular alteration: According to Eustis et al (1990), in the F344 rat, Foci of cellular alteration, adenoma and carcinoma are thought to represent a spectrum of lesions that constitute the natural history of neoplasia." (p. 78) Goodman et al treats "hepatocellular alteration" as possibly pre-neoplastic

a) In the opinion of the PWG, when an adenoma is down-graded to "hepatocellular alteration", is this finding now of no more concern in addressing carcinogenicity than normalcy, or should it be viewed as a preneoplastic lesion?

b) To the extent that an adenoma is downgraded to "hepatocellular alteration", does this suggest the latter lesion carries more weight than under previous criteria as preneoplastic, and likewise, to the extent carcinoma is downgraded to adenoma, does adenoma now have more associated concern than under previous criteria?

c) To what extent does implementation of new criteria represent anything more than a frame shift among endpoints recognized as constituting the natural history of neoplasia?

d) The PR Notice calls for all cellular alteration, adenomas and carcinomas to be examined by the peer review pathologist. The PWG reread does not provide incidence values for hepatocellular alteration since only some of these (moderate and severe) were referred to PWG. Since this lesion is a part of the natural history of neoplasia, their incidences should be determined (for consistency) by the same PWG as the one that identified adenoma and carcinoma.

e) Since the PWG employed "current" criteria (Goodman et al 1994) for diagnosing the liver lesions, which include specific criteria for hepatocellular alteration (with no reference to severity) as well as for adenoma and carcinoma, shouldn't all such diagnoses of any of these lesions made prior to the PWG should be subject to confirmation by the PWG.

f) Although we have several PWG diagnoses of hepatocellular alteration–without severity indicated–the report does not permit an accurate statistical analysis of this important aspect of the neoplastic process. If we use only the incidences of these lesions appearing in the final PWG report as definitive for hepatocellular alterations, there is but one in the control with several in dose groups, leading only to the interpretation there is no NOEL in the study for this hepatocellular effect, which carries added weight as evidence of a neoplastic process.

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April 10, 2000

Dr. William Burnam
Chief, Science Analysis Branch
Health Effects Division
Mail Code 7509C
USEPA
Washington, DC 20460

Dear Bill:

Please find enclosed a copy of the questions that were faxed to me last Friday, April 7, 2000. I have indicated our response to each of the questions in bold. The responses have been reviewed by each of the PWG participants and have received their concurrence. Hopefully, this will answer many of the questions included in the fax. I will be at EPA at 9:00 a.m. on Tuesday, April 11, 2000, to answer any questions that may remain. Judy Hauswirth and Meena Sonawane from Jellinek, Schwartz and Connolly, Inc., sponsors of the PWG, will also attend the meeting.

As I indicated on Friday, I am a participant in the ILSI sponsored workshop on Developmental Neurotoxicity. I am part of the pathology break out group in this workshop. It is scheduled to begin at 10:00 a.m. on Tuesday. By providing my written responses ahead of time, hopefully I will still be able to attend the ILSI Workshop as scheduled. I have, however, informed the Workshop Organizers that I might be slightly late due to an unscheduled meeting.

We look forward to our meeting with you, Marion and Brian. My flight is scheduled to arrive from Raleigh at 8:06 a.m. and I will come directly from the airport to your office.

Sincerely,

JERRY F. HARDISTY, D.V.M.
PWG Chairperson

JFH:amm
Enclosures

ATTENTION: Jerry Hardisty

April 10, 2000

The following questions were the result of a discussion between Drs. Brian Dementi, Marion Copley and William Burnam (HED/OPP/EPA) regarding the Jellinek, Schwartz and Connolly, Inc. letter to Patricia Moe (March 20, 2000), their summary and the attached malathion PWG report for female rat livers.

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Questions regarding the PWG

1. **Differences** - The Jellinek, Schwartz and Connolly, Inc. letter to Patricia Moe (March 20, 2000) (includes Summary and Significance Results of .....) has several differences from their attached Pathology Working Group (report dated March 17, 2000).

a) The Summary states that "Dr. Henry Bolte, the original study pathologist, was present at the PPR," while the PWG report itself makes no mention of Bolte's presence at the PPR. Was Dr Bolte present and if Dr. Bolte was present on March 14, what was his role?

Response: Dr. Bolte was present at Huntingdon Life Sciences, East Millstone, NJ, on March 14, 2000. Dr. Bolte is a staff pathologist at HLS and served as the host for Drs. Busey and Hardisty. He had no role in the Peer Review. As the Study Pathologist, he made himself available to address any questions and retrieved any additional slides, reports or tissues which were requested.

b) The Summary states in item 3. "The PWG was conducted in full compliance with the procedures described in PR Notice 94-5 (August 24, 1994). All slides containing sections previously diagnosed by the SP or the PPR as hepatocellular carcinoma or adenoma or as indicating **varying degrees of severity** (emphasis added) of non-neoplastic proliferative lesions (foci of cellular alteration and or hypertrophy/hyperplasia) were examined." (p. 1) By contrast, the PWG report says "hepatocellular alteration of **moderate degree of severity** (emphasis added) diagnosed by either the study pathologist or reviewing pathologist were submitted to the PWG." (p. 10) What was actually read by the PWG, all cellular alterations or only those diagnosed as of moderate severity or above by either the SP or PPR?

Response: The PWG report is correct. The PWG reexamined the liver from all female rats with hepatocellular carcinoma, hepatocellular adenoma, hepatocellular hypertrophy/hyperplasia (regenerative), or moderate degrees of severity of hepatocellular alteration diagnosed either during the initial examination by the Study Pathologist or during the peer review by the Reviewing

Pathologist.

2. **Process questions**

a) The PWG abstract states, "The purpose of the PWG review was to determine the incidence of hepatocellular neoplasms in female rats following currently accepted nomenclature and diagnostic criteria." Do the pathologists' signatures on the PWG report endorse the contents of the entire report including the discussion of the historical control database and the other signs of toxicity in the study in question OR do they just refer to the consensus report comparisons of the slides read by the PWG?

Response: The draft PWG report was completed involving input from all members of the PWG panel on March 15, 2000 immediately following the reexamination of the slides. Their signatures indicate that they endorse the contents of the PWG report. The information concerning the historical control database and the other signs of toxicity in the study were included in the PWG report as referenced from the original study report. This information was discussed by the PWG and was included in the PWG report to provide support for the PWG's conclusions.

b) Did the chairperson complete the following after the peer review on 3/14/2000: 1) examined all slides that were positive, 2) have recuts made of two blocks, and 3) organized materials including coding slides for PWG on the 3/15/2000?

Response: In preparation for the PWG to be held on March 15, 2000, on March 14, 2000 the PWG Chairperson did the following:

- **Examined the Individual Animal Gross Pathology findings from all female rats to identify female rats with a gross observation of nodule(s)/mass(es) noted at necropsy. The histopathology data were then reviewed from these animals to determine if a corresponding microscopic diagnosis was reported for these animals. Gross observations of nodule(s)/mass(es) were present for three Group I, four Group II, one Group IV and five Group V female rats. No correlating microscopic lesions appeared to be present for nodule(s)/mass(es) noted grossly for one Group IV female, 4531, and one Group V female, 5512. The wet tissues were reexamined by the PWG Chairperson, Dr. Hardisty, and the Study Pathologist, Dr. Bolte. The untrimmed lesions were sectioned by Dr. Bolte and the sections were processed and microscopic slides were prepared of the sections for examination by the PWG on March 15, 2000.**

- **On March 14, 2000 the PWG Chairperson identified the animal numbers of all female rats with a diagnosis of hepatocellular carcinoma, hepatocellular adenoma, hepatocellular**

hypertrophy/hyperplasia (regenerative), or moderate degrees of severity of hepatocellular alteration diagnosed either during the initial examination by the Study Pathologist or during the peer review by the Reviewing Pathologist.

- **These animal numbers were randomized by group and then coded using ascending numbers for each animal examined by the PWG. All sections of liver from these animals were reexamined by the PWG. Additionally, the slides prepared from Group IV female, 4531, and Group V female, 5512, were coded for examination by the PWG.**

c) A peer review was performed on all slides. Is this review available to EPA upon request?

Response: A separate Peer Review report is not required and was not prepared. A computerized Pathology Peer Review program operating on a laptop computer was used to record the Reviewing Pathologist's comments. Slide Review Worksheets were generated to identify the animals that would be examined by the PWG. The final PWG report includes the Reviewing Pathologist's comments resulting from the peer review. The reviewing pathologist's Slide Review Worksheets are not generally submitted to the agency. Copies of the Reviewing Pathologist's Slide Review Worksheets are present at EPL and copies of them could be provided to the EPA upon request.

d) Can a reviewing pathologist reliably evaluate as many as an estimated 625 sections in one day?

Response: Slides were reexamined from 15 female control and 15 Group V female rats from the interim sacrifice and from 55 female rats per group (5 groups) from the terminal sacrifice. Except for those with gross observations of nodule(s)/mass(es), each animal had two sections of liver mounted on one glass slide. In most cases the spleen was also mounted on the same slide as the liver. Exceptions involved cases with mononuclear cell leukemia with exceptionally large spleens. In these cases, the spleen was mounted on a different slide. Nodule(s)/mass(es) were reported grossly for the liver of three Group I, four Group II, one Group IV, and five Group IV females. This resulted in the reexamination of approximately 30 slides from the interim sacrifice and approximately 288 slides from the terminal sacrifice. The total number of slides examined during the peer review was approximately 318 slides.

EPL has been conducting pathology peer reviews for over 20 years for the NCI/NTP Bioassay Testing Program and for commercial clients. We have developed an efficient approach to these reviews which includes using

technicians to arrange the slides in the exact order to be examined. The reviewing pathologist uses a computerized peer review software program (PQA®) developed by EPL to record all comments using a portable computer. The original study pathologist's findings are transferred electronically to the PQA program resulting in a paperless peer review. These efficiencies allow the reviewing pathologist to concentrate on the slide review without the necessity of referring to the study pathologist's report or handwriting the review results. During a typical peer review of a study the reviewing pathologist can easily examine 500-600 slides per day.

e) What was the function of the chairman during the PWG reread?

Response: The PWG Chairperson is responsible for the organization and conduct of the PWG and for preparation of the PWG's report. During the PWG's reexamination of the slides, the PWG Chairperson records the final opinions of the PWG for each of the coded animals. Following the PWG review of the slides the PWG Chairperson uncodes the findings and presents the results to the panel for their discussion and interpretation.

f) What was the level of review (by the peer review pathologist) of all of the slides?

Was the evaluation made with the intent to just confirm the diagnoses of the study pathologist and identify any missed diagnoses; or did he independently determine his own diagnoses for all slides? Was his evaluation blind or did he have access to the existing diagnoses and the groups that the animals belonged to.

Response: A peer review is not done in a coded manner. The primary purpose the peer review conducted prior to a PWG review is to reexamine all slides of the organ being reviewed to identify any additional lesions which should be examined by the PWG and to confirm the findings reported by the study pathologist.

g) The PR notice states that "the Agency should be provided with a detailed pathology report, which presents the PWG findings and includes the original diagnosis and the new diagnosis of each slide read, and a comment column to note any discrepancies…" Is there a reason a comment column was not provided to explain where the differences came from?

Response: PR Notice 94-5 states that, "the Agency should be provided with a detailed pathology report , which represents the PWG findings and includes the original diagnosis and the new diagnosis for each slide read, and a comment column to note any discrepancies, missing slides, etc."

A comments column was not included in the tabulation of the PWG findings

in Appendix A of the PWG report. This was an oversite on the part of the PWG Chairperson. No missing slides were noted during the review and the only discrepancies in the data noted during the review involved two untrimmed gross lesions discussed above (see response to 2.b.). Differences of opinion in diagnosis between the study pathologist's initial diagnoses and the PWG consensus diagnoses are included in the tabulation in Appendix A. These differences resulted from the reexamination of the slides using the criteria included on pages 14 and 15 of the PWG report.

3. **Technical questions**

a) Since hepatocellular alterations, hypertrophy/hyperplasia, adenoma and carcinoma constituted the focus of this PWG, and all of these end points play a role in characterizing the neoplastic response as discussed in Goodman, why was there no mention of the hepatocellular alterations in the discussion about carcinogenicity? The PWG addressed the interpretation of the adenoma and carcinomas in this study. Since hepatocellular alteration together with adenoma and carcinoma are said to constitute the natural history of neoplasia—although not required—it would have been informative to have the PWG's input into the interpretation based on incidences of all three of these end points.

Response: The purpose of the PWG review was to determine the incidence of hepatic neoplasms in female rats following currently accepted nomenclature and diagnostic criteria (Goodman DG, et al, 1994) . Slides with diagnoses of hepatocellular hypertrophy/hyperplasia (regenerative) or moderate degrees of severity of hepatocellular alteration were examined by the PWG to determine if any of these diagnoses may have been neoplastic lesions. The PWG was not asked to address the significance, if any, of the nonneoplastic findings in the liver.

b) In the case of a Group II rat #2554, a Group IV rat, #4531 and a Group V rat, #5528, both Drs. Bolte and Busey identified adenoma, while PWG reported "no corresponding diagnosis"; are we to assume they saw nothing, or perhaps a severe form of hepatocellular alteration?

Response: In all three cases the PWG considered the lesions diagnosed by the Study and Reviewing Pathologists as adenoma to be a focus of hepatocellular alteration. The PWG Consensus Diagnosis corresponding to the adenoma was tabulated as no corresponding diagnosis because the Study Pathologist had also diagnosed hepatocellular alteration for these animals and the PWG Consensus Diagnosis was recorded across from this finding.

c) If new criteria of classification are being applied in this study of malathion, what does the panel say regarding relevance of the historical control database, from both NTP and the testing facility?

Response: Historical control data are used to determine if the concurrent control group is within the expected range considered to be normal for the species, strain, sex and age used as the test animal in bioassay studies. The new criteria have no affect on the relevance of the NTP historical control data since that data have been peer reviewed using the same criteria for classification as used during this PWG examination of liver tumors in female F344 rats from the malathion study. It is not known if the testing facility's historical control database has been peer reviewed. Although the exact criteria used for the classification of liver tumors in the testing facilities historical control database is not known, the criteria published by Goodman, et al., 1994 and Eustis, et al., 1990 have generally become the industry standard. It is unlikely that significant changes would be made to the testing facilities historical control data even if reviewed using the criteria published by Goodman, et al. The only way to know for sure would be to conduct a reexamination of the liver tumors reported in the female rats in the testing facility's historical control database using this criteria.

d) What was seen microscopically by the PWG that led to changes of diagnoses first rendered by Drs. Bolte and Busey? Also, to what extent do these revisions depend upon Goodman et al (1994)? There was no comment column detailing what the differences in diagnoses were due to.

Response: The changes in the diagnoses first rendered by Drs. Bolte and Busey resulted from the PWG's examination of each of the lesions and the group's discussion of the morphologic features of the lesions as they relate to the criteria published by Goodman, et al. The comment column is intended to document discrepancies, missing slides, etc., rather than detail differences in opinion in diagnosis due to the application of diagnostic criteria. No missing slides were noted during the review and the only discrepancies in the data noted during the review involved two untrimmed gross lesions discussed above (see response to 2.b.).

e) Is one section of liver adequate to render a definitive diagnosis when there is a debate and change of diagnosis? In many cases Drs. Bolte and Busey were in agreement, but the diagnosis was changed by PWG, does this suggest that the lesions were borderline (e.g., between alteration-adenoma, adenoma-carcinoma)?

Response: A single section of a liver lesion is adequate to render a definitive diagnosis. In any study, lesions that are borderline in nature are often present. It is in these cases, that the value of the PWG examination is most obvious. This provides a forum where the slides can be examined and reexamined and the detailed features of the lesions can be discussed by a panel of experienced experts in order to render the most appropriate diagnosis for each lesion examined and therefore provides a high level of confidence in the final

diagnosis.

3. Cellular alteration: According to Eustis et al (1990), in the F344 rat, Foci of cellular alteration, adenoma and carcinoma are thought to represent a spectrum of lesions that constitute the natural history of neoplasia." (p. 78) Goodman et al treats "hepatocellular alteration" as possibly pre-neoplastic

Response: Eustis et al., 1990 states that: "Experimental models suggest that some foci may be precursors of hepatocellular neoplasms. However, only a very small proportion of the foci progress to neoplasms even with continued administration of the chemical. When the carcinogenic stimulus is removed, many foci and neoplastic nodules regress. Factors that influence progression or regression of foci are unclear."

Goodman, et al., 1994 states that: "Foci of cellular alteration occur spontaneously in aging rats, and certain types may reach close to 100% incidence in certain strains even though the incidence of hepatocellular neoplasms is very low, i.e. F344 females. The incidence, size, and/or multiplicity of foci usually are increased, and time of development decreased by the administration of hepatocarcinogens. Moreover, foci generally precede the development of tumors, and they have been categorized as preneoplastic lesions. However, they are reversible lesions and there is conflicting evidence regarding the biological nature of the rat liver foci. Some studies indicate foci represent an early stage in neoplastic development and that at least some have the capacity to progress to tumors. Other studies indicate that, although induced by carcinogens, foci may be nonneoplastic end-stage lesions. Evidence recently has been presented that not all foci may be related to carcinogenesis. The later study was a review of F344 rat livers from a limited number of carcinogenicity tests in the National Toxicology Program (NTP) Archives."

a) In the opinion of the PWG, when an adenoma is down-graded to "hepatocellular alteration", is this finding now of no more concern in addressing carcinogenicity than normalcy, or should it be viewed as a preneoplastic lesion?

Response: In the opinion of the PWG, "hepatocellular alteration" should be regarded as a nonneoplastic proliferative lesion of the liver. It should not be regarded as a preneoplatic lesion unless there is evidence of progression from hepatocellular alteration to neoplasia which was not evident in this study.

b) To the extent that an adenoma is downgraded to "hepatocellular alteration", does this suggest the latter lesion carries more weight than under previous criteria as preneoplastic, and likewise, to the extent carcinoma is downgraded to adenoma, does adenoma now have more

associated concern than under previous criteria?

Response: The degree of concern must be related to what is considered to be the biological nature of the lesion being considered. Hepatocellular alteration is a nonneoplastic proliferative lesion of the liver and should not be considered a preneoplastic lesion in this study. Hepatocellular adenomas are considered to be benign liver tumors and hepatocellular carcinomas are considered to be malignant liver tumors. .

c) To what extent does implementation of new criteria represent anything more than a frame shift among endpoints recognized as constituting the natural history of neoplasia?

Response: The implementation of new criteria is based on the results of years of research and discussion concerning the biological nature of proliferative lesions in the liver. It represents much more that a frame shift among endpoints recognized as constituting the natural history of neoplasia. New criteria are established and implemented so that the diagnoses of proliferative lesions better reflect the biological behavior of the lesion. This provides a better basis for risk assessment when considering neoplastic endpoints.

d) The PR Notice calls for all cellular alteration, adenomas and carcinomas to be examined by the peer review pathologist. The PWG reread does not provide incidence values for hepatocellular alteration since only some of these (moderate and severe) were referred to PWG. Since this lesion is a part of the natural history of neoplasia, their incidences should be determined (for consistency) by the same PWG as the one that identified adenoma and carcinoma.

Response: The purpose of the PWG review was to determine the incidence of hepatocellular neoplasms in female rats following currently accepted nomenclature and diagnostic criteria. In most instances, hepatocellular alteration in the F344 rat is not considered to represent a continuum with hepatic neoplasia. In this study there was no evidence that hepatocellular alteration was increased in incidence with treatment or related to the neoplasms observed in this study. The PWG did not reexamine all cases of hepatocellular alteration diagnosed by the study pathologist. They did, however, confirm the presence of hepatocellular alteration initially reported by the study pathologist when present on the slides examined. To examine trends in the incidence of hepatocellular alteration the Agency should refer to the data provided in the final study report.

e) Since the PWG employed “current” criteria (Goodman et al 1994) for diagnosing the liver lesions, which include specific criteria for hepatocellular alteration (with no reference to severity) as well as for adenoma and carcinoma, shouldn’t all such diagnoses of any of these

lesions made prior to the PWG should be subject to confirmation by the PWG.

Response: Refer to response given for the 3.d. above.

f) Although we have several PWG diagnoses of hepatocellular alteration–without severity indicated–the report does not permit an accurate statistical analysis of this important aspect of the neoplastic process. If we use only the incidences of these lesions appearing in the final PWG report as definitive for hepatocellular alterations, there is but one in the control with several in dose groups, leading only to the interpretation there is no NOEL in the study for this hepatocellular effect, which carries added weight as evidence of a neoplastic process.

Response: The purpose of the PWG review was to determine the incidence of hepatocellular neoplasms in female rats following currently accepted nomenclature and diagnostic criteria. The PWG did not focus on lesions diagnosed as hepatocellular alteration initially by the study pathologist. They examined lesions diagnosed as moderate degrees of severity of hepatocellular alteration diagnosed either during the initial examination by the Study Pathologist or during the peer review by the Reviewing Pathologist. There was no intent by the PWG to indicate the severity of the hepatocellular alteration. The NTP currently recommends that foci of cellular alteration be diagnosed only as present in Carcinogenesis Bioassays currently being conducted without an indication of severity.

In most instances, hepatocellular alteration in the F344 rat is not considered to represent a continuum with hepatic neoplasia. In this study there was no evidence that hepatocellular alteration was increased in incidence with treatment or related to the neoplasms observed in this study. If statistical analysis of incidence and severity of hepatocellular alteration is performed, the incidence and severity of hepatocellular alteration included in the final study report should be used.

It would be erroneous to use only the incidences of these lesions appearing in the final PWG report as definitive for hepatocellular alterations since the PWG was not asked to examine the liver from all female rats with a previous diagnosis of hepatocellular alteration. Since the only purpose of the PWG was to confirm the incidence of hepatocellular neoplasms in female rats, no conclusions can be made concerning the incidence of hepatocellular alteration using only the data presented in the PWG report.

The data in the PWG report can only be used to determine the NOEL for hepatocellular neoplasms. The PWG report cannot be used to determine the NOEL for hepatocellular alteration. The Agency must refer to the final study report to determine the NOEL for hepatocellular alteration (see following table).

Attachment 2

Incidence of Hepatocellular Alteration Reported by the Study Pathologist in the Final Report for Study No. 90-3341: A 24-Month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration (Daly IW, 1996)

Group No.	I	II	III	IV	V
Interim Sacrifice Female Rats					
Liver (No. Examined)	(15)	(0)	(0)	(0)	(15)
Hepatocellular Alteration: Basophilic	1	0	0	0	0
Hepatocellular Alteration: Eosinophilic	0	0	0	0	0
Hepatocellular Alteration: Clear Cell	0	0	0	0	0
Terminal Sacrifice					
Liver (No. Examined)	(55)	(55)	(55)	(55)	(55)
Hepatocellular Alteration: Basophilic	23	29	23	16	4
Hepatocellular Alteration: Eosinophilic	1	0	2	0	1
Hepatocellular Alteration: Clear Cell	0	0	1	0	0

MEMORANDUM

TO: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

FROM: John M. Pletcher, DVM, MPH, DACVP
Consulting Pathologist
Cancer Assessment Review Committee
Health Effects Division

DATE: 28 March, 2000

SUBJECT: Review of Pathology Working Group (PWG) conduct and findings for Proliferative Lesions of the Liver in Female Rats in a 24-Month Oral Toxicity/Oncogenicity Study of Malathion

I have reviewed the documents you provided concerning the recent Pathology Peer Review (PPR) and Pathology Working Group (PWG) of proliferative liver lesions in female rats that were part of a 24 month oral toxicity and oncogenicity study of Malathion. The PWG was conducted on March 15 and the PPR on the day or days before March 15. (Question 1) I have determined that the PPR and PWG were conducted in accordance with industry standards and in compliance with procedures set forth in PR Notice 94-5. The Peer Review Pathologist, Dr. Bill Busey, is an experienced rodent pathologist as are all of the members of the Pathology Working Group (Drs. Hardisty, Hildebrandt, Garman and Elwell). (Question 2) I have complete confidence in the consensus diagnoses produced by this PWG. I contacted one of the PWG members and asked him why the carcinoma diagnoses made by the Study Pathologist (Dr. Bolte) were overturned by the PWG. He told me that the working group members were nearly unanimous in their opinions, particularly so concerning changing the carcinoma diagnoses. It was his opinion that the Study Pathologist, being without the aid of another pathologist to consult with while doing the initial evaluation, used incorrect criteria. (Question 3) Differences between the Study Pathologist's diagnoses and those of the PWG do not, in my opinion, require extensive explanation. It is the purpose of a PWG to correct incorrect diagnoses that arise from the use of wrong or outdated diagnostic criteria, or for any other reason; the consensus opinion of the PWG is by far the stronger data. (Question 4) Knowing personally or by reputation most of the members of this particular PWG, I do not hesitate to accept their opinion that the lesions diagnosed by the Study Pathologist as carcinomas are actually adenomas and the down-grading of some adenomas to foci of cellular alteration. (Question 5) Concerning the sufficiency of time for the PPR pathologist to do his review, it would take an experienced pathologist no more than a minute to evaluated two sections of rat liver; the sections with proliferative lesions would, of course, require a minute or two more. The review could have been completed in one day. (Question 6) It would be ideal for the Study Pathologist to sit with the PPR pathologist at a double-

head microscope during the review, but not necessary to assure a quality review. (Question 7) As to the desirability of another pathologist reviewing the changed lesions, I am confident that it would serve no purpose; as stated above, the consensus diagnoses of a properly conducted PWG carries far more weight than an individual opinion. (Question 8) The question of distinguishing hepatocellular carcinoma from adenoma is, of course, pertinent. Nothing in the biological sciences is absolute; however, well-defined criteria have recently been published by the Society of Toxicologic Pathologists (STP Guides) making the differentiation of carcinomas from adenomas relatively easy for a pathologist with rodent experience. (Question 9) The suggestion of making and evaluating additional sections of the lesions whose diagnoses were changed is problematic in that such an action would statistically bias the study data. (Question 10) Concerning the diagnosis of adenoma vs a focus of cellular alteration, although the STP Guides establish criteria for each, this can be a problem area. Both lesions can compress adjacent hepatic parenchyma, although an adenoma typically does so much more than a focus, and can be composed of similar appearing cells forming a proliferative lesion of similar size. Clearly, there is some overlapping here and one person's adenoma may be another's focus of cell alteration. Again, a consensus opinion of experienced pathologists is the strongest data, i.e. the opinion of a properly constituted and conducted PWG.

The above referenced PPR and PWG were properly conducted and the PWG's participating members are veterinary pathologists with a great deal of rodent pathology experience. The report is thorough and well documented. It is my opinion that the conclusions presented in the report are well founded and valid, and it is my recommendation that they be accepted by the Cancer Assessment Review Committee.

Attachment 5

March 27, 2000

MEMORANDUM

SUBJECT: A Pathology Working Group Review of Liver Slides from the 24-month Toxicity/Oncogenicity Study in the Rat

FROM: Brian Dementi
TB1, HED (7509C)

THROUGH: Marion Copley
RAB1, HED (7509C)

TO: Sanju Diwan, Executive Secretary
Carcinogen Assessment Review Committee, HED (7509C)

Cheminova A/S has submitted to the Agency (through Jellinek, Schwartz & Connolly, Inc.), a letter including: A Pathology Working Group Review of Liver Slides from the 24-month Toxicity/Oncogenicity Study in the Rat. The cover letter is dated March 20, 2000 and the report is dated March 17, 2000. A MRID has not yet been assigned to the PWG report.

HED is requesting that this report be reviewed by veterinary pathologists, Drs. Brenneke and/or Pletcher, with particular attention to the issues identified below. Due to the short time frame to the CARC meeting for this issue, the response needs to be back by March 30, 2000, if at all possible. In order to expedite our receipt or your feedback, an advance copy of your response could be submitted by either FAX or E-MAIL followed by an official response.

Questions to be considered in review of PWG report:

1) Do you feel the PWG adequately followed the procedures set forth in PR Notice 94-5?

2) Can we rely on the PWG consensus tumor incidences?

3) Does the PWG report adequately address differences between and among the Study Pathologist, the Reviewing Pathologist and the PWG consensus findings?

4) Please express any views you may have on the down grading of all carcinomas to adenomas in the various dose groups? Similarly, the downgrading of certain adenomas to non-tumor status?

5) Please comment on the question of whether one day is sufficient time for the Reviewing Pathologist to examine in the expected manner all of the slides in question, i.e. two sections of liver from each of 305 animals, plus sections of macroscopic lesions.

6) Should the study pathologist have been present during the day the Reviewing Pathologist performed his review?

7) Do you recognize any need for the Agency's pathologist to examine the slides for which diagnoses were revised as a result of the PWG?

8) Please offer comment on the level of difficulty of distinguishing hepatocellular carcinoma from hepatocellular adenoma.

9) In those cases wherein the original diagnosis has been revised, should additional sections of liver be taken?

10) In the case of rat number 4514 where the diagnosis of hepatocellular adenoma, concurred in by the Study Pathologist and the Reviewing Pathologist, was revised at the PWG meeting to hepatocellular alteration, what does this mean in terms of parameters characterizing this lesion, i.e., is there a fine line distinction between the two, and how likely is it that yet another pathologist would identify the lesion as an adenoma. Please bear in mind that for regulatory purposes, the adenoma designation would be considered positive, while the hepatocellular alteration may have no more impact than that of a liver absent any finding.

FOR YOUR INFORMATION ONLY:

1) We recognize that the PWG report employs incorrect denominators (70 as opposed to 55 animals) for tumor incidence calculations in the control and high dose groups in Table 1 (p. 1). The seventy includes 15 low and high dose animals that were sacrificed at 1 year.

2) Any discussion of regulatory implications will be taken up at the CARC meeting, e.g. the PWG's claim that dosing in the high dose group was excessive. So please do not discuss the regulatory implications of this report. This will be discussed at the CARC meeting, at which time the pathologist's opinion would be desired.

EPA Reviewers: Sanjivani Diwan *Signature*
RRB4, Health Effects Division (7509C) *Date*

Branch Senior Scientist: Alberto Protzel *Signature*
TB1, Health Effects Division (7509C) *Date*

DATA EVALUATION RECORD
Supplement to the Original DER (HED Doc. 013822)

Study Type: Combined Chronic/Oncogenicity Study; OPPTS 870.4300 [83-5]

DP BARCODE: D264571 Submission No.: S529758
P.C. CODE: 057701 Tox. Chemical No.: 535

Test Material (purity): Malathion; butanedioic acid, [(dimethoxyphosphinothioyl) thio] diethyl ester (97.1% a.i.)

Synonym: Mercaptosuccinic acid diethyl ester; S-ester with 0, 0, -dimethyldithiophosphate

Citation: Ira W. Daly, Ph.D., D.A.B.T., 27 February 1996. A 24-Month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration. Huntingdon Life Sciences, East Millstone, NJ, Study No. 90-3641, MRID 43942901, Unpublished.

Hardisty, J. F. (2000). Pathology Working Group (PWG) Peer Review of Proliferative Lesions of the Liver in Female Rats In a 24-Month Oral Toxicity/Oncogenicity Study of Malathion (MRID 43942901)," performed by Experimental Pathology Laboratory, Inc., RTP, NC; dated March 17, 2000. EPA Product No. 297-006, MRID 45069401, Unpublished.

Sponsor: Cheminova Agro A/S, P.O. Box 9, DK-7620, Lemvig, Denmark

Executive Summary: In a combined chronic toxicity/oncogenicity study (MRID 43942901), malathion (97.1% a.i.) was administered to 90 Fischer 344 rats/sex/dose via the diet for up to 24 months at dose levels of 0, 100/50 (100 ppm for first 3 months of study, 50 ppm for duration of study in both sexes due to finding of erythrocyte cholinesterase inhibition in females only at 3 month assay) 500, 6,000 or 12,000 ppm [equivalent to respective mean values of 0, 4, 29, 359 and 739 mg/kg/day (males) and 0, 5, 35, 415 and 868 mg/kg/day (females)].

The only clinical sign observed was yellow anogenital staining among females at 12000 ppm. **Increased mortality was seen in females at 12000 ppm and in males at 500, 6000 and 12000**

ppm. All 12000 ppm males died or were sacrificed moribund by about 94 weeks. Treatment related decrements in body weight gain were observed at 6000 and 12000 ppm in both sexes. **Food consumption was increased at 100 ppm in males for the first 3 months (prior to lowering of dose to 50 ppm).** At subsequent time points for males, and across all time points for females food consumption was increased, the LOAEL = 6000 ppm and NOAEL = 500 ppm. Among parameters for hematology, erythrocyte count was reduced in males at 12000 ppm, mean corpuscular hemoglobin concentration was decreased in males at 6000 and 12000 ppm; and the following were observed in rats of both sexes at 6000 and 12000 ppm: increased platelet count, decreased mean corpuscular volume and mean corpuscular hemoglobin. Hence, for hematologic parameters overall, LOAEL = 6000 ppm, NOAEL = 500 ppm, both sexes. Among clinical chemistry parameters, erythrocyte cholinesterase inhibition, males, LOAEL = 6000 ppm, NOAEL = 500 ppm; females, at 3 months, the enzyme was inhibited at all doses, LOAEL = 100 ppm. After 3 months, when lowest dose was reduced to 50 ppm, LOAEL = 500 ppm, NOAEL = 50 ppm. For plasma cholinesterase inhibition, males, LOAEL = 500 ppm, NOAEL = 50 ppm (100 ppm first 3 months); females, LOAEL = 6000 ppm, NOAEL = 500 ppm. For brain cholinesterase inhibition, LOAEL = 6000 ppm, NOEL = 500 ppm, both sexes. For inhibition of cholinesterase activity, for males the overall NOAEL is 50 ppm (4 mg/kg/day) and the LOAEL is 500 ppm (29 mg/kg/day) based on inhibition of plasma activity at 24 months. For females the overall (beyond 3 months) NOAEL is 50 ppm (5 mg/kg/day) and the LOAEL is 500 ppm (35 mg/kg/day) based on inhibition of erythrocyte activity. Decreased aspartate aminotransferase, females, 12000 ppm; decreased alkaline phosphatase, males and females, 6000 and 12000 ppm; elevated blood urea nitrogen, males, 12000 ppm; elevated cholesterol, males and females, 6000 and 12000 ppm; elevated gamma-glutamyl transpeptidase, males and females, 6000 and 12000 ppm. Ocular effects testing inconclusive. Organ weight effects: increased kidney and liver weights, males and females, 6000 and 12000 ppm; thyroid/parathyroid weight increased (males), decreased (females) 6000 and 12000 ppm; increased spleen weight, males, 6000 and 12000 ppm; increased heart weight, males, 6000 ppm (term). In males, increases in liver and thyroid/parathyroid weights may have extended to 500 ppm. **Microscopic findings: non-neoplastic**: nasal mucosa and nasopharynx (several pathologies), males and females, 6000 and 12000 ppm; bilateral subacute-chronic inflammation/chronic nephropathy (high incidence in all study groups including controls), increased severity, males, 6000 and 12000 ppm, females, 500, 6000 and 12000 ppm; stomach (several pathologies), males and females, 6000 and 12000 ppm; increased incidence parathyroid hyperplasia, males and females, all doses; other findings in various tissues (thyroid, lymph nodes, lungs, liver, spleen, adrenal gland, eyes) as summarized in the review, being more remarkable in males, and often extending across the top three doses in males and top two doses in females; **neoplastic:** there was no treatment related increase in liver tumors in **male rats**. In **female rats** (based on the Pathology Work Group Re-Read), there was a positive trend ($p = 0.005$) for adenomas. The incidence of adenomas was significantly increased by pair-wise comparison at 12,000 ppm (5/38, 13%, $p = 0.009$) when compared to controls (0/41). There were no carcinomas in any group. When compared to the historical control data of the testing laboratory, the incidences of adenomas at 12,000 ppm (13%) exceeded the historical control range (0 to 5%) and mean (1.6%). In addition, the incidence of adenomas exceeded the historical control incidence (adenomas, 0.44%) of the NTP (1998 report).

The CARC also determined that hepatocellular alteration did not appear to indicate progression to neoplasia. The Committee concluded that a) although the increased incidence

of liver tumors in female rats only occurred at an excessively toxic dose (12,000 ppm), it provided evidence of carcinogenicity because the incidence was statistically significant by pair-wise comparison; there was a statistical trend; and the incidence was outside the range of both the testing facility and NTP historical control data bases; b) there was the presence of a few rare tumors, oral palate mucosa in females and nasal respiratory epithelium in male and female Fischer 344 rats. With the exception of one nasal and one oral tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion. These tumors can not be distinguished as either treatment related or due to random occurrence. However, the CARC determined that recut would not alter their conclusion.

This study is classified as Acceptable and satisfies the guideline requirement for a chronic/carcinogenicity study in rats (83-5).

This Executive Summary supersedes the previous summary for MRID 43942901(HED Doc. 013822) . The new summary has been modified based on the PWG re-read of pathology data (HED Doc. 013720 and a memorandum dated April 25, 2000) as well as HIARC (HED Doc. 013820) and CARC deliberations.